inspection is made or (ii) a colorimetric assay, CIE L*, a*, b* (or CIELAB), which is more quantitative than the BY system. However, in either case, color assessment between multiple samples should be normalized against protein concentration in order to assure a meaningful assessment. For example, referring to Example 9 below, the Protein A eluate has a "b*" value of around 2.52 which corresponds to a BY value of approximately BY5 (when measured at a concentration of 5 g/L protein in the protein A eluate). If the color of the Protein A eluate is to be compared to another sample, then the comparison should be made against the same protein concentration. The b* value in the CIELAB color space is used to expresses coloration of the samples and covers blue (−) to yellow (+). The higher the b* value of a sample is compared to another indicates a more intense yellow-brown coloration in the sample compared to the other.

[0015] In one embodiment, aflibercept is produced from a host cell genetically engineered to express aflibercept using CDM. In one aspect, other species or variants of aflibercept are also produced. These variants include aflibercept isoforms that comprise one or more oxidized amino acid residues collectively referred to as oxo-variants. A clarified harvest sample produced using CDM comprising aflibercept as well as its oxo-variants can be subjected to a capture chromatography procedure. In one aspect, the capture step is an affinity chromatography procedure using, for example, a Protein A column. When a sample extracted from an affinity eluate, which may or may not manifest a yellow-brown color, is analyzed using, for example, liquid chromatography-mass spectrophotometry (LC-MS), one or more oxidized variants of aflibercept may be detected. Certain amino acid residues of a modified aflibercept are shown to be oxidized including, but not limited to, histidine and/or tryptophan residues. In one aspect, the variants can include oxidation of one or more methionine residues as well as other residues, see infra.

[0016] In another aspect, the variants can include oxidation of one or more tryptophan residues to form N-formylkynurenine. In a further aspect, the variants can include oxidation of one or more tryptophan residues to form mono-hydroxyl tryptophan. In a particular aspect, the protein variants can include oxidation of one or more tryptophan residues to form di-hydroxyl tryptophan. In a particular aspect, the protein variants can include oxidation of one or more tryptophan residues to form tri-hydroxyl tryptophan.

[0017] In another aspect, the variants can include one or more modifications selected from the group consisting of: deamidation of, for example, one or more asparagines; one or more aspartic acids converted to iso-aspartate and/or Asn; oxidation of one or more methionines; oxidation of one or more tryptophans to N-formylkynurenin; oxidation of one or more tryptophans to mono-hydroxyl tryptophan; oxidation of one or more tryptophans to di-hydroxyl tryptophan; oxidation of one or more tryptophans to tri-hydroxyl tryptophan; Arg 3-deoxyglucosonation of one or more arginines; removal of C-terminal glycine; and presence of one or more non-glycosylated glycosites.

[0018] In another embodiment, the invention is directed to methods for producing aflibercept. In one aspect, a clarified harvest sample comprising aflibercept and its variants are subjected to a capture step such as Protein A affinity chromatography. Subsequent to the affinity step, an affinity eluate can be subjected to ion exchange chromatography. The ion exchange chromatography can be either cation or anion exchange chromatography. Also contemplated to be within the scope of the present embodiment is mixed-mode or multimodal chromatography as well as other chromatographic procedures which are discussed further below. In a particular aspect, the ion exchange chromatography is anion exchange chromatography (AEX). Suitable conditions for employing AEX include, but are not limited to, Tris hydrochloride at a pH of about 8.3 to about 8.6. Following equilibration using, for example, Tris hydrochloride at a pH of about 8.3 to about 8.6, the AEX column is loaded with sample. Following the loading of the column, the column can be washed one or multiple times using, for example, the equilibrating buffer. In a particular aspect, the conditions used can facilitate the differential chromatographic behavior of aflibercept and its oxidized variants such that a fraction comprising aflibercept absent significant amounts of oxo-variants can be collected in a flowthrough fraction while a significant portion of oxo-variants are retained on the solid-phase of the AEX column and can be obtained upon stripping the column—see Example 2 below, FIG. **11**. Referring to FIG. **11** and Example 2, changes in oxo-variants can be observed between the different production steps. For example, this change can be illustrated by data in the "Tryptophan Oxidation Level (%)" section, specifically, the "W138(+16)" column. There it can be observed that the oxo-variants (specifically, oxo-tryptophan) went from about 0.131% in a load sample to about 0.070% in a flowthrough sample following AEX chromatography (AEX separation 2), indicating that there was a reduction in oxo-variant of aflibercept using AEX.

[0019] Use of ion exchange can be used to mitigate or minimize color. In one aspect of the present embodiment, a clarified harvest sample is subjected to capture chromatography, for example, using Protein A affinity chromatography. The affinity column is eluted and has a first color with a particular BY and/or b* value assigned thereto. This Protein A eluate is then subjected to ion exchange chromatography such as anion exchange chromatography (AEX). The ion exchange column is washed and the flowthrough is collected and has a second color having a particular BY and/or b* value assigned thereto. In a particular aspect, the color value (either "BY" or "b*") of the first color differs from the second color. In a further aspect, the first color of the Protein A eluate has a more yellow-brown color as compared to the second color of the AEX flowthrough as reflected by the respective BY and/or b* value. Typically, there is a reduction in yellow-brown color of the second color following AEX when compared to the first color of the Protein A eluate. For example, the use of anion exchange reduced the yellow-brown color observed in a Protein A eluate sample from a b* value of about 3.06 (first color) to about 0.96 (second color) following AEX—see Example 2, Table 2-3 below.

[0020] In one aspect of the embodiment, the pH of both the equilibration and wash buffers for the AEX column can be from about 8.30 to about 8.60. In another aspect, the conductivity of both the equilibration and wash buffers for the AEX column can be from about 1.50 to about 3.00 mS/cm.

[0021] In one aspect of the embodiment, the equilibration and wash buffers can be about 50 mM Tris hydrochloride. In one aspect, the strip buffer comprises 2M sodium chloride or 1N sodium hydroxide or both (see Table 2-2).